

## A COMPARATIVE EVALUATION OF THE EFFECT OF REMINERALIZING AGENTS ON THE SURFACE MORPHOLOGY AND MICROHARDNESS OF BLEACHED ENAMEL AN INVITRO STUDY

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### ABSTRACT

#### Aim

*To evaluate the effect of remineralizing agents on the surface morphology and microhardness of bleached enamel. Methodology*

*Forty maxillary anterior teeth whose crowns were mounted on resin blocks with the labial surface facing upwards. Baseline microhardness was recorded. All the specimens were subjected to three cycles of bleaching for 3 minutes at interval of 24 hours per cycle. The post bleach specimens were subjected to SEM and Vickers microhardness tester. The specimens are divided into four groups based on the remineralizing agent used, Group I: no remineralizing agent applied (control); Group II: sodium fluoride foaming solution applied; Group III: CPP-ACP + Fcrème applied; Group IV: Calcium Nanophosphate paste applied. Specimens were subjected to SEM and Vickers microhardness test.*

#### Results

*The observations were then statistically analyzed using One Way ANOVA followed by Student's 't' test to detect significance level of  $p \leq 0.05$  between different groups. Group IV showed higher VHN values compared to other groups which were comparable to values of unbleached enamel microhardness. SEM evaluation demonstrated more remineralization of bleached enamel surface in Group IV.*

#### Conclusions

*McInnes bleaching solution decreased the microhardness of enamel. All the remineralizing agents used increased the microhardness of bleached enamel.*

**KEYWORDS:** Remineralization, Microhardness, CPP-ACP+F, Sodium Fluoride Solution, Calcium Nanophosphate Paste

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### INTRODUCTION

Dental enamel is a crystalline lattice network composed of various minerals, the principal component of which is a complex calcium phosphate mineral called hydroxyapatite.

In the oral cavity, changes in the mineral content of the teeth regularly occur. Under normal conditions, the loss and gain balance out<sup>1</sup>. However, if the balance shifts towards demineralization which can occur for a

number of reasons like increased frequency of acid exposure like in bleaching tends to alter the total demineralization / remineralization amounts, resulting in significantly greater amount of mineral loss<sup>2</sup> that may transmit heat, cold, pressure and pain more readily than normal enamel.

Bleaching has become a popular esthetic procedure as it represents the most conservative treatment option for discolored teeth. The effects of bleaching on enamel are probably related to their pH causing alterations in the mineral composition and microhardness<sup>3,4</sup>. To counteract the deleterious effects of bleaching agents on enamel mineral content, various remineralizing agents have been introduced recently into the market, which are of great interest in research now.

The aim of the present in vitro study was to evaluate the effect of 2% Sodium Fluoride solution (Oral-B Neutra Foam)), CPP-ACP+F crème (GC Tooth Mousse Plus, Recaldent), Calcium Nanophosphate paste (Desensibilize Nano-P, FGM Produtos Odontologicos) with artificial saliva acting as control on the surface morphology and microhardness of the human enamel after exposed to McInnes bleaching solution.

## MATERIALS AND METHODS

Forty freshly extracted human permanent maxillary anterior teeth were selected that were free of dental caries or restorations and were of normal crown anatomy. The teeth were stored in artificial saliva at room temperature until testing was done.

### Preparation of the Samples

The crowns of the teeth were separated at cemento-enamel junction using a slow speed diamond disc under water spray. Then the enamel specimens were mounted in self curing acrylic resin (DPI) such that the labial surface was impregnated in the cold cure acrylic resin facing upwards. Using plastic moulds, the resins were made into blocks (6mmx4mm) and polished using 1000 grit carborandum paper discs (Z Zlinker) under water spray to produce flat surfaces. The prepared specimens were stored in artificial saliva to prevent dehydration<sup>5</sup>. Base line microhardness recorded using Vickers Hardness Tester (Matsuzawa – Seiki co-ltd, Tokyo, Japan, Model No. MHT-1).

### Preparation of the Bleaching Solution

McInnes bleaching solution consists of a mixture of 1 ml of 36% hydrochloric acid, 1 ml of 30% hydrogen peroxide and 0.2 ml of anesthetic ether which was mixed in the ratio of 5:5:1. The mixture is prepared freshly in a dappen dish before each application<sup>6</sup>.

All the specimens were subjected to three cycles of bleaching (each cycle of bleaching at an interval of 24 hours) with McInnes solution for 3 minutes each time using a cotton applicator. Demineralized surface was observed under Scanning Electron Microscope (CX-100S, Wonkyung International Co. Ltd) before application of remineralizing agent at 1000x and 4000x and also subjected for microhardness test using Vickers microhardness tester Tester (Matsuzawa – Seiki co-ltd, Tokyo, Japan, Model No. MHT-1).

Then all the specimens were divided randomly into 4 groups based on the remineralizing agent used.

**Group I:** Specimens were subjected to bleaching and stored in artificial saliva acting as a control.

**Group II:** Specimens were subjected to bleaching followed by application of 2% Sodium Fluoride solution (Oral-B Neutra Foam) on the bleached enamel surface.

**Group III:** Specimens were subjected to bleaching followed by application of CPP-ACP crème (GC Tooth Mousse, Recaldent) on the bleached enamel surface.

**Group IV:** Specimens were subjected to bleaching followed by application of Calcium Nanophosphate paste (Desensibilize Nano-P, FGM Produtos Odontologicos) on the bleached enamel surface.

Remineralizing agent was applied on the respective samples as grouped with cotton applicator on the post bleached enamel samples everyday for 7 days with minimum application time of 3 minutes and these samples were washed under deionized water and then stored in artificial saliva for 7 days.

Prior to microhardness measurement, three specimens were randomly selected from each group for surface morphology evaluation under Scanning Electron Microscope (CX-100S, Wonkyung International Co. Ltd) at magnification 1000x and 4000x.

### **Surface Hardness Measurement**

Microhardness measurements of top surfaces of the specimens after application of remineralizing agents in respective groups were determined by Vickers hardness testing machine. The Vickers surface microhardness test method consisted of indenting the test material with a diamond tip, in the form of a right pyramid with a square base and Vickers microhardness readings were undertaken using a load of 50g for 15 seconds. All hardness values were expressed in vickers hardness, where  $1HV=1.854 P/d^2$ , with P being the indentation load and d the diagonal length. As the Vickers microhardness tester was very sensitive to operational procedures, an average reading of three indentations were taken to avoid operator bias.

The tabulated observations were then statistically analyzed using Analysis of variance technique (One Way ANOVA) followed by Student's 't' test to detect significance level of  $p \leq 0.05$  between different groups using software SPSS 18.

## **RESULTS**

The mean and standard deviation were calculated for each group. The mean VHN of bleached samples showed reduction in microhardness of enamel. Statistically significant difference was found between post bleaching and samples subjected to remineralizing agents used in respective groups. However, Group IV- Calcium Nanophosphate paste (Desensibilize Nano-P, FGM Produtos Odontologicos) showed higher VHN values compared to other groups which were comparable to values of unbleached enamel microhardness. Group I (Control) showed the least VHN values.

Specimens were analyzed using scanning electron microscope (SEM) and the morphology of the enamel surface was observed.. Group I (Control) showed pronounced morphologic surface alterations like partial removal of the aprismatic layer, increased depth of enamel grooves, exposure of enamel prisms, craters and shallow erosions. In Group IV- Nanohydroxyapatite, SEM photomicrographs revealed a surface not completely smooth but uniformity of the aprismatic surface layer could be observed.

## **DISCUSSIONS**

The increased demand for oral health and esthetics led to a greater number of patients seeking cosmetic dental procedure, particularly dental bleaching as discoloured teeth negatively interfere with the harmony of the smile.

Dental bleaching represents a popular conservative treatment modality or may become an auxiliary therapy when restorative procedures are required to eliminate colour abnormalities<sup>7</sup>.

It is speculated that the reaction between the bleaching agent and the organic/inorganic content of enamel can result in morphological alterations<sup>4</sup>.

The deleterious effects of the bleaching agents on the dental hard tissues vary and are related to agent composition and concentration<sup>8,9</sup>, prescribed use instructions, time of exposure, pH values and type of solutions used<sup>10,11,12</sup>. The surface alterations or defects that could be detected on the surface of enamel are increased porosity<sup>12,13</sup> and precipitate deposition characterised enamel erosion, depression with crater formation<sup>14</sup>, removal of the aprismatic layer and the exposure of the enamel prisms could also be detected<sup>13,15</sup>.

To neutralize the ill effects of bleaching (reduction in micro-hardness due to loss of calcium and phosphate ions), various agents have been used like baking soda, prophylactic paste containing fluoride and use of copious amount of water<sup>5</sup>.

In addition to inhibition of the deleterious effects of bleaching agents on enamel mineral content, the benefits of using remineralizing agents in bleaching agents or after bleaching could include the reduction of enamel solubility and reduced sensitivity due to mineral deposition in enamel crystallites<sup>16</sup>.

So the present in-vitro study is done to evaluate the effect of three different remineralizing agents on surface morphology and microhardness of bleached enamel surface.

In this study, Mc Innes bleaching agent was selected as it is the most commonly used bleaching agent in vital bleaching procedures. McInnes bleaching technique was superficially recommended for the treatment of teeth exhibiting endemic dental fluorosis because of its superficial nature, easy manipulation and its quality of being less expensive when compared to other commercially available agents like carbamide peroxide<sup>17,18</sup>.

Mc Innes solution used may promote changes in micromorphology, chemical composition and microhardness of enamel after bleaching treatment<sup>12,14,19</sup>.

To simulate the clinical situation, non-carious human teeth were used and stored in artificial saliva. Studies have shown that no alterations in intact enamel microhardness observed, when stored in artificial saliva<sup>20</sup>.

In Group I, maximum demineralization of enamel surface observed as the specimens were stored in artificial saliva after bleaching the enamel surface without application of remineralizing agent. This acts as a control.

In group II, the specimens were remineralized with 2% Sodium Fluoride solution (Oral-B Neutra Foam). The use of fluoride to remineralize the tooth structure post bleaching is well documented. The calcium fluoride layer formed on enamel inhibits demineralization or a reduction in microhardness values<sup>20</sup>.

In group III, the specimens were remineralized with CPP-ACPF crème (GC Tooth Mousse Plus, Recaldent). The casein phosphopeptides(CPP)- a milk protein contents multi phosphorseryl sequence with the ability to stabilize calcium phosphate in nanocomplexes in solution like amorphous calcium phosphate(ACP). Through their multiple phosphorseryl sequences CPP binds to ACP in meta stable solution preventing the dissolution of calcium and phosphate ions. CPP-ACP also acts as reservoir of bio-available calcium and phosphate and maintains the solution in super saturated state, thus facilitating remineralisation. Fluoride in CPP-ACPF is presumed to have a synergistic effect on remineralizing

action<sup>19,21,22,23</sup>.

In group IV, the specimens were remineralized with Calcium Nanophosphate paste (Desensibilize Nano-P, FGM Produtos Odontologicos). Group IV showed highest microhardness which was statistically significant when compared to other groups. SEM micrographs showed progressive microparticle deposition with interprismatic and prismatic enamel structures completely hidden by a thick homogeneous layer, probably due to hydroxyapatite nanocrystals<sup>24</sup>.

The calcium nanophosphate crystals may have penetrated more deeply into the defects of the enamel, forming a “reservoir-like” deposit of the eroded calcium and phosphate ions. The reservoir-like deposit help maintain a state of supersaturation with enamel minerals<sup>25</sup>.

Further, the Fluoride concentration in nanophosphate paste is 10 times higher (9000ppm) than that of CPP-ACP Plus crème (900ppm). This higher concentration may affect the remineralization process<sup>24</sup>.

The amount of demineralization of enamel by bleaching was assessed using a microhardness tester<sup>5</sup>. The Vickers hardness number is the quotient obtained by dividing the KgF load by the square mm area of indentation. When the mean diagonal of the indentation has been determined the Vickers hardness may be calculated from the formula  $HV = 1.854 F/d^2$ . Vickers test is easier to use than other hardness tests since the required calculations are independent of the size of the indenter, and the indenter can be used for all materials irrespective of hardness.

## CONCLUSIONS

Within the limitations of the current study, it was concluded that

McInnes bleaching solution decreased the microhardness of enamel.

All the remineralizing agents used increased the microhardness of bleached enamel.

Group IV Calcium Nanophosphate paste (Desensibilize Nano-P, FGM Produtos Odontologicos) showed higher microhardness values compared to Group II -2% Sodium Fluoride solution (Oral-B Neutra Foam) and Group III -CPP-ACP+F (GC Tooth Mousse Plus, Recaldent) which was equivalent to the original microhardness of unbleached enamel. SEM evaluation demonstrated more remineralization of bleached enamel surface in Group IV when compared to other groups.

However, the results of this in vitro study may not be directly related to the clinical situation, hence further in vivo studies are recommended to substantiate the results of this present study.

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## APPENDICES

### ANNEXURE

**Table 1: Vickers Microhardness Values in Kg/Mm<sup>2</sup> of the Bleached Enamel Surface before and after Application of Bleaching Agent and after Application of Remineralizing Agents with in Groups**

Group	Before Application of Bleaching Agent	After Application of Bleaching Agent	After Application of Remineralizing Agent
<b>GROUP I</b>	79.45	64.13	64.05
<b>GROUP II</b>	79.84	65.27	70.98
<b>GROUP III</b>	79.26	65.38	74.12
<b>GROUP IV</b>	79.53	66.92	78.39

**Table 2: Mean, Standard Deviation and Test of Significance of Microhardness within Different Groups**

S. No	Variable	Groups Compared	Mean	SD	P-Value*	Significant Groups at 5% Level
1.	<b>MICROHARDNESS BEFORE BLEACHING</b>	GROUP I	79.45	1.78	0.24 (NS)	-
		GROUP II	79.84	1.80		
		GROUP III	79.26	1.79		
		GROUP IV	79.53	1.79		
2.	<b>MICROHARDNESS AFTER BLEACHING</b>	GROUP I	64.13	1.14	.85 (NS)	-
		GROUP II	65.27	1.16		
		GROUP III	65.38	1.16		
		GROUP IV	66.92	1.15		
3.	<b>MICROHARDNESS AFTER APPLICATION OF REMINERALIZING AGENT</b>	GROUP I	64.05	1.14	< 0.05 (Sig)	IV>III>II>I
		GROUP II	70.98	1.52		
		GROUP III	74.12	1.69		
		GROUP IV	78.39	1.76		

Statistically significant level at P<0.05; S: significant; NS: Not significant.

\* One –way ANOVA was used to calculate the p-value



**Table 3: Change in Microhardness Values before Bleaching to Post Bleaching and 7 Days after Remineralization within Groups**

**GROUP I**

S. No	Variable	Mean	SD	't' Value *	p-value Significance*
1.	Baseline microhardness to post bleaching microhardness	71.79	1.46	08.643	<0.05(Sig)
2.	Post bleaching microhardness to 7 days of remineralization	64.09	1.14	06.241	0.67(NS)

Statistically significant level at  $P < 0.05$ ; S: significant; NS: Not significant.

\* One –way ANOVA was used to calculate the p-value

\*Student's 't' test was used to calculate the t-value for inter variable comparison

**GROUP II**

S. No	Variable	Mean	SD	't' Value *	p-value Significance*
1.	Baseline microhardness to post bleaching microhardness	72.32	1.47	09.751	<0.05(Sig)
2.	Post bleaching microhardness to 7 days of remineralization	69.75	1.42	11.962	<0.05(Sig)

Statistically significant level at  $P < 0.05$ ; S: significant; NS: Not significant.

\* One –way ANOVA was used to calculate the p-value

\*Student's 't' test was used to calculate the t-value for inter variable comparison

**GROUP III**

S. No	Variable	Mean	SD	't' Value *	p-Value Significance*
1.	Baseline microhardness to post bleaching microhardness	72.55	1.48	08.923	<0.05(Sig)
2.	Post bleaching microhardness to 7 days of remineralization	68.12	1.34	10.054	0.16(NS)

Statistically significant level at  $P < 0.05$ ; S: significant; NS: Not significant.

\* One –way ANOVA was used to calculate the p-value

\*Student's 't' test was used to calculate the t-value for inter variable comparison



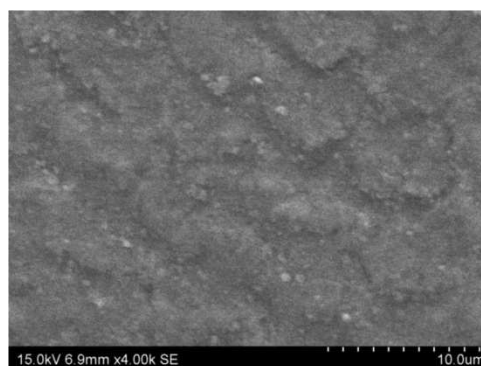
**GROUP IV**

S. No	Variable	Mean	SD	't' Value *	p-value Significance*
1.	<b>Baseline microhardness to post bleaching microhardness</b>	73.22	1.47	08.954	<0.05(Sig)
2.	<b>Post bleaching microhardness to 7 days of remineralization</b>	72.65	1.45	13.241	<0.001(HS)

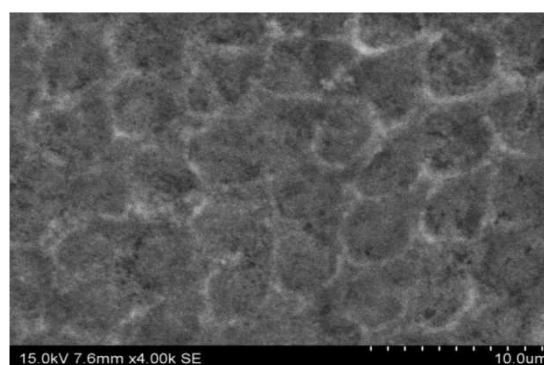
Statistically significant level at  $P < 0.05$ ; S: significant; HS: Highly significant

\* One –way ANOVA was used to calculate the p-value

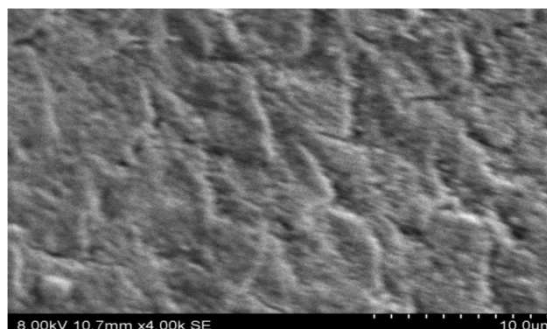
\*Student's 't' test was used to calculate the t-value for inter variable comparison



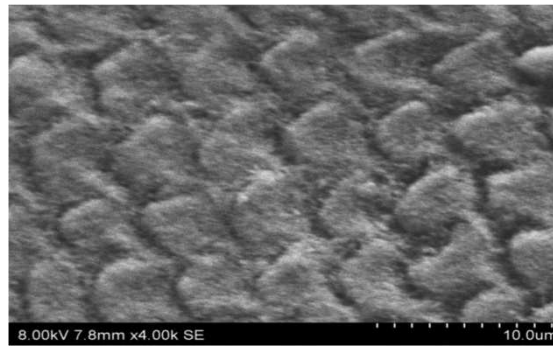
**Group I: Control**



**Group II: Sodium Fluoride Foaming Solution**



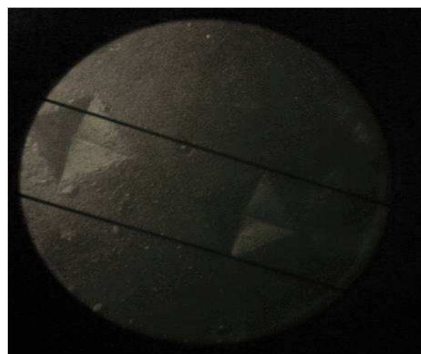
**Group III: CPP+ACP+F CRÈME**



**Group IV: Calcium Nanophosphate Paste**



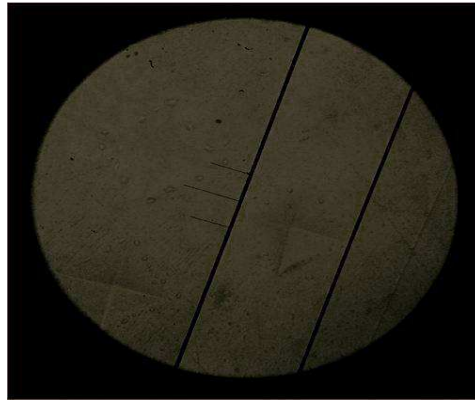
**Group I: Control**



**Group II: Sodium Fluoride Foaming Solution**



**Group III: CPP+ACP+F CREME**



**Group IV: Calcium Nanophosphate Paste**

